Docket No: 4-30598/A Sys-2068

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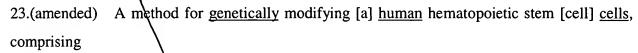
and adeno-associated viral vectors; and

(b) obtaining modified human hematopoietic stem cells.

19.(amended) The method according to claim 18, further comprising culturing the hematopoietic stem cells in the presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 200 ng/mL.



20. (amended) The method according to claim 19, further comprising culturing the hematopoietic stem cells in the presence of a interleukin 3 (IL3) in a concentration range of about 5 ng/mL to about 200 ng/mL.



(a) contacting a [gene delivery vehicle] <u>vector</u> comprising a polynucleotide sequence <u>encoding a heterologous gene</u> with a population of hematopoietic stem cells cultured in the presence of an effective amount of a thrombopoietin ligand (TPO), a flt3 ligand (FL), and interleukin 6 (IL6) <u>each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL</u>,

wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors; and

(b) obtaining modified human hematopoietic stem cells.



24.(amended) The method of claim 23, further comprising culturing the stem [cell] cells in the presence of an effective amount of leukemia inhibitory factor (LIF) wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL

25.(amended) The method of claim 23, further comprising culturing the stem [cell] cells in the

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presence of an effective amount of interleukin 3 (IL3) wherein the effective amount is in the range of about 10 ng/mL to about 100 ng/mL.

26.(amended) The method of claim 23, further comprising culturing the stem [cell] cells in the presence of a c-kit ligand wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL

27.(amended) The method of claim 25, further comprising culturing the stem [cell] <u>cells</u> in the presence of a c-kit ligand <u>wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.</u>

Please delete claims 1 - 17, 21, 22, 29, and 30.

Please add the following new claims:

- 52.5
- 31. The method according to claim 23, wherein the effective amount of TPO and FL individually is in the range of about 5 ng/mL to about 200 ng/mL and the effective amount of IL6 is in the range of about 10 ng/mL to about 100 ng/mL.
- 32. The method according to claim 23, wherein the vector is a retroviral vector.
- 33. The method according to claim 23, wherein the heterologous gene is a marker gene.
- 34. The method according to claim 23, further comprising expanding the modified human hematopoietic cells.
- 35. The method according to claim 23, wherein the human hematopoietic cell is a CD34⁺Thy-1⁺

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Lin cell.

- 36. The method according to claim 23, further comprising culturing the hematopoietic stem cells in the presence of fibronectin or BetronectinTM.
- 37. A method of transducing mammalian CD34⁺ hematopoietic cells including a subpopulation of hematopoietic stem cells comprising,
 - (a) obtaining a source of hematopoietic cells including the subpopulation of hematopoietic stem cells;
 - (b) culturing said cells with the cytokines TPO, FL and IL-6, individually provided in the range of about 0.1 ng/mL to about 500 ng/mL;
 - (c) infecting the cultured cells with a retroviral vector including a polynucleotide sequence encoding a heterologous gene; and
 - (d) obtaining transduced cells wherein said gene is expressed.
- 38. The method according to claim 37, wherein the TPO, FL and IL-6 are individually provided in the range of about 5 ng/mL to about 200 ng/mL.
- 39. The method according to claim 37, further comprising culturing the cells in the presence of an effective amount of leukemia inhibitory factor (LIF) wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.
- 40. The method according to claim 37, further comprising culturing the cells in the presence of an effective amount of IL-3 wherein said effective amount is in the range of 10 ng/mL to about 100 ng/mL.
- 41. The method according to claim 39, further comprising culturing the cells in the presence of